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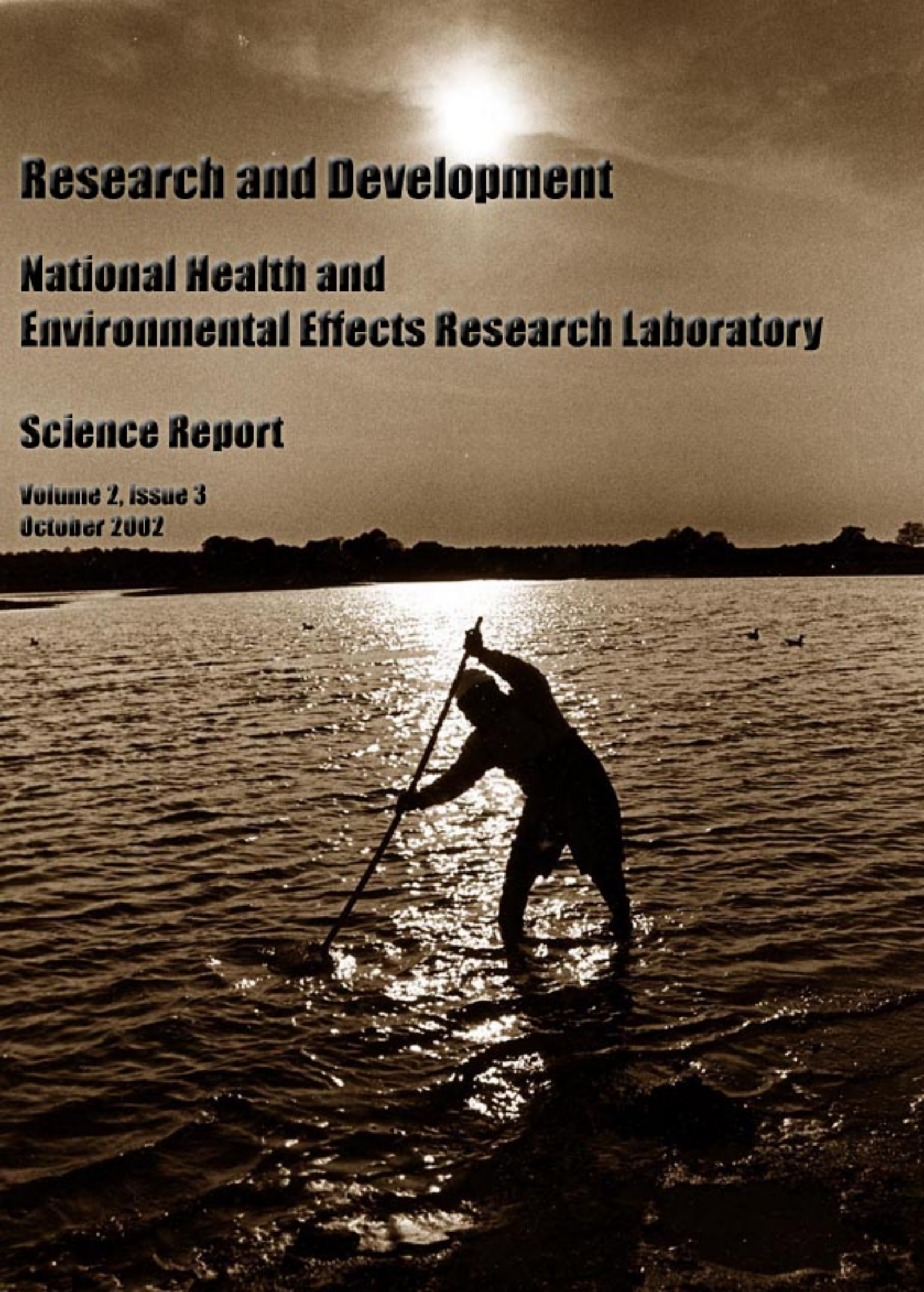

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RESEARCH

Most Midwestern Amphibians Are at Low Risk for UV Effects

Exposure of amphibians to ultraviolet radiation (UVR) has been suggested as a cause of declines in populations and increases in the presence of hindlimb malformations. Dr. Gerald Ankley, Dr. Stephen Diamond, Joseph Tietge, Gregory Peterson, Gary Holcombe, Kathleen Jensen, David DeFoe, and Ryan Patterson of the Mid-Continent Ecology Division of the National Health and Environmental Effects Research Laboratory (NHEERL), with colleagues from the University of Minnesota's Natural Resource Research Institute, compared levels of UVR exposure in 26 midwestern wetlands with levels that caused adverse effects in controlled outdoor experiments on three midwestern amphibian species: northern leopard frogs (*Rana pipiens*), green frogs (*R. clamitans*), and mink frogs (*R. septentrionalis*). Assuming that the wetlands in the study accurately represent all northern midwestern wetlands, the results suggest that most amphibians in the region currently are at low risk for UVR effects.

This research is the first to use a risk assessment approach to address the question of whether UVR harms amphibians in nature. The results contribute to understanding the role of changing UVR levels in wetlands and provide perspective on other hypotheses suggested to explain reductions in amphibian numbers, such as pesticide exposure, disease, parasitism, and natural causes.

Three consecutive papers were published in the July 1, 2002, issue of *Environmental Science and Technology (ES&T)* under the umbrella title of "Assessment of the Risk of Solar Ultraviolet Radiation to Amphibians." In "Part I. Dose-Dependent Induction of Hindlimb Malformations in the Northern Leopard Frog (*Rana pipiens*)," Dr. Ankley and co-authors present UVR threshold levels derived from controlled outdoor experiments. In "Part II. In Situ Characterization of Solar Ultraviolet Radiation in

Amphibian Habitats," Gregory Peterson and colleagues survey factors that control the UVR dose in 26 northern Minnesota and Wisconsin wetlands. In "Part III. Prediction of Impacts in Selected Northern Midwestern Wetlands," Dr. Diamond's group compares the experimental UVR effects levels with estimates of UVR exposure for the 26 wetlands.

The authors conclude that currently there is a slight risk of amphibian mortality, and a somewhat higher risk of malformation, in 2 of the 26 wetlands studied. Risk of malformation was also apparent in one additional wetland. However, factors such as climate change, animal behavior, and variation among wetlands can alter exposure levels, suggesting that UVR should not be ignored in future consideration of possible effects on amphibians and other aquatic organisms. [*ES&T*, 2002, 36:2853-2858; 36:2859-2865; 36:2866-2874].

Fecal Bacterium Causes Coral White Pox Disease

Massive outbreaks of coral diseases over the last several decades may be causing the substantial declines in the biodiversity and abundance of reef-building corals. The greatest losses on Caribbean and South Florida reefs have been among the branching elkhorn and staghorn corals, *Acropora palmata* and *Acropora cervicornis*. A paper recently published in the *Proceedings of the National Academy of Science (PNAS)* details the destruction of elkhorn coral populations, with losses in the Florida Keys typically in excess of 70%. *Serratia marcescens*, a common intestinal bacterium, was identified as the causal agent of white pox. Kathryn Patterson, a University of Georgia, Athens, doctoral student in marine sciences, was the principal investigator. She conducted the research at the Gulf Ecology Division under the mentorship of co-author Dr. Deborah Santavy.

Serratia marcescens is a ubiquitous bacterium found in human feces, but it is more commonly associated with hospital infections in immune-compromised patients.

This species is also found as intestinal microbiota of other animals and as a free-living microbe in water and soil. *Serratia* species are known to cause disease in both marine and freshwater fishes and to pose a serious threat as an opportunistic pathogen to other marine organisms. Sewage may be the source of *S. marcescens* contamination. The *S. marcescens* strain (PDL100) isolated from white pox-affected elkhorn coral may also be associated with pollution of fecal origin. At present, however, the origin, pathogenic mechanisms, and host range of the PDL100 strain are unknown but under investigation.

EPA, the State of Florida, and the National Oceanic and Atmospheric Administration have developed a Water Quality Protection Program (WQPP) for the Florida Keys National Marine Sanctuary. Findings from this research will help implement the WQPP and provide a rationale for improved treatment of wastewater and stormwater in the Florida Keys. The study builds on the knowledge that corals are highly sensitive to all forms of pollution. A new no-discharge zone designation now in effect for all state waters within the Sanctuary will help curtail the dumping of harmful waste by boats and ships, a key priority of the Sanctuary's Water Quality Protection Program. [*PNAS*, 2002, 99:8725-8730].

Pfiesteria shumwayae **Kills Fish by Predation, Not Poisoning**

Pfiesteria are dinoflagellates, a type of microscopic algae implicated in certain fish kills. Scientists at the Virginia Institute of Marine Sciences of the College of William and Mary showed that *Pfiesteria shumwayae* kills fish by feeding directly on their skin, not by releasing a potent toxin into the water. EPA is a contributor to the multi-agency program that supported this research. Dr. Calvin Walker of the Gulf Ecology Division is a co-author who worked on research design issues related to toxins. Earlier researchers attributed several massive fish kills in mid-Atlantic estuaries and some human health effects to toxins released in the water by *Pfiesteria piscicida*, a very similar dinoflagellate, but the specific toxins were not identified.

Three different approaches showed how *Pfiesteria shumwayae* kills fish. (1) Using a *P. shumwayae* culture known to kill fish, *Pfiesteria* dinoflagellates were removed from the culture by filtration and centrifugation, presumably leaving behind any secreted toxins in the aqueous layer. When larval fish were added to the aqueous layer, none was killed. (2) Fish kills were observed only when the larval fish were put into direct physical contact with *Pfiesteria* dinoflagellates in water. (3) Using videomicrography, high-resolution microscopy, and electron microscopy, large numbers of *Pfiesteria* dinoflagellates were seen swarming toward the larval fish, attaching to them, and feeding directly on their skin--in effect, skinning the fish alive. Thus, *Pfiesteria shumwayae* is shown to kill fish by direct predation, not by release of toxins. Much more research is needed before the effects of *Pfiesteria* on human health can be explained in similar detail. [*Nature*, 2002, Aug. 5 issue on-line; 418:967-970].

Drinking Water Disinfection By-Product Causes Cancer in Rats

A lifetime exposure study in rodents, described in a recent paper by Michael George, Donald Doerfler, Tanya Moore, Stephen Kilburn, and Dr. Anthony DeAngelo of the Environmental Carcinogenisis Division (ECD) and colleagues at Pathology Associates, Inc., found that bromodicholoromethane (BDCM), a major by-product of chlorine disinfection of drinking water, is carcinogenic in the male F344/N rat.

A previous study by the National Toxicology Program found that BDCM administered orally in corn oil increased kidney and colon cancer in male F344/N rats and kidney cancer in male $B6C3F_1$ mice. These NTP findings are significant because epidemiology studies point to an increased risk of colorectal cancer in humans drinking chlorinated surface waters, and kidney cancer is rare in male mice.

When ECD administered BDCM in drinking water to male F344/N rats and male $B6C3F_1$ mice, there was no increase in the incidence of large cell adenocarcinoma in the colon of the rat or kidney cancer in the mouse, contrary to the NTP results using

corn oil. However, because of the increased prevalence and multiplicity of liver neoplasms, BDCM administered in drinking water was judged to be carcinogenic in the male F344/N rat. Because the dose-response curve was complicated by a bimodal shape that can be only partially explained by an inhibition of BDCM metabolism in the liver, additional work is needed to understand the underlying cause more fully.

These results can be used in the further development of a risk assessment for BDCM, as required by the Safe Drinking Water Act. (See also the following article on BDCM metabolism.) [*International Journal of Toxicology*, 2002, 21:219-230].

Metabolism of Water Disinfection By-Product Studied in Rat and Human Liver

Bromodichloromethane (BDCM), an important by-product of the drinking water disinfection process, has toxic and carcinogenic effects in rodents at concentrations much higher than those found in drinking water supplies. The Experimental Toxicology Division (ETD) has developed a physiologically-based pharmacokinetic (PBPK) model for BDCM in rats, which potentially can be extended to a biologically-based dose-response model to extrapolate effects from rats to humans. A PBPK model predicts the absorption of a chemical and its distribution to organs in a whole animal. To provide essential information for developing the extrapolation model, Dr. John Allis, recently retired from ETD, and Dr. Guangyo Zhao, an ETD post-doctoral fellow in the Curriculum in Toxicology at the University of North Carolina at Chapel Hill, recently published two papers that identified and quantitatively measured the important pathways for metabolism of BDCM in the liver of both rats and humans.

Previous work in ETD revealed that several liver cytochrome P450(CYP) enzymes, especially CYP2E1, are responsible for the vast majority of BDCM metabolism in the rat. The scientists measured the kinetics of BDCM metabolism for each CYP enzyme involved with metabolism of drugs and other foreign compounds in rat and human liver. Metabolic activities of the important CYP enzymes were measured

one at a time by two *in vitro* methods. The first used preparations made by recombinant DNA techniques that produced a single CYP enzyme so that its activity could be measured without interference from others. Both rat and human CYP enzymes were tested. Because, unlike in rats, there is no information on BDCM metabolism in humans, a second approach was used for the human CYP enzymes. In this case, microsomes isolated from human livers were tested, with the activity of one CYP enzyme measured while blocking the activity of the others with inhibitory antibodies. Microsomes give a closer approximation to the whole animal than do the recombinant enzymes.

Recombinant enzyme measurements confirmed that the three CYP enzymes identified in the rat were indeed the most important ones. In both rats and humans, CYP2E1 was dominant at low BDCM concentrations, but two other enzymes were important at moderate BDCM concentrations. The measurements in microsomes confirmed these conclusions. Although CYP2E1 is the most important BDCM-metabolizing enzyme in both rats and humans, there are important differences between the species for the other enzymes. These results are essential to the development of a model to extrapolate effects from rats to humans. (See also the previous article on BDCM carcinogenesis.) [*Chemico-Biological Interactions*, 2002, 140:137-153; 140:155-168].

Drinking Water Disinfection By-product Decreases Fertility in Male Rats

Bromochloroacetic acid (BCA) is one of the more prevalent disinfection by-products (DBPs) formed when disinfectants are used to treat drinking water. In a recent publication from the Reproductive Toxicology Division, Dr. Gary Klinefelter, Lillian Strader, Juan Suarez, and Naomi Roberts described the effects of BCA on reproduction in male rats. BCA adversely affected both the formation of normal sperm and the fertility of treated male rats, as well as altered the level of SP22, a sperm membrane protein identified earlier in Klinefelter's laboratory.

Adult male rats were treated with BCA orally for 14 days, using a range of doses from 8 to 240 mg/kg/day. Various sperm parameters and the level of fertility of the treated rats were then measured. Fertility of the treated animals was also compared with the level of SP22, a protein found on the surface of sperm. Both the fertility of sperm and their SP22 level were decreased in all dose groups, setting the Lowest Observed Adverse Effect Level at 8 mg/kg. Importantly, the decrease in SP22 was correlated with the decrease in fertility.

Further work is underway to establish a No Observed Adverse Effect Level for the effects of BCA on sperm. Current efforts are also focused on the effects of mixtures of several of the known water DBPs such as BCA, dibromoacetic acid, and dibromochloroacetic acid. Finally, an epidemiology study, in collaboration with the University of North Carolina at Chapel Hill, is assessing the effects of water containing various levels of DBPs on the quality of sperm from men drinking the water. SP22 levels and other parameters will also be evaluated. [*Toxicological Sciences*, 2002, 68:164-173].

Work Continues on Endocrine Disruptor Screening Program

In response to a mandate by the Food Quality Protection Act and to growing concerns that environmental chemicals may adversely affect human health by altering the endocrine system, EPA launched an endocrine disruptor screening program (EDSP). Three NHEERL Divisions–Gulf Ecology (GED), Mid-Continent Ecology (MED), and Reproductive Toxicology (RTD)–continue work on the Tier 1 and Tier 2 assays that are likely to be included in the final testing battery. Tier 1 involves screening tests to select endocrine-active chemicals for further study; Tier 2 involves detailed tests to determine the mode of action of the chemicals selected in Tier I.

Dr. William Benson, Director of GED, is Acting Chair of the Endocrine Disruptor Methods Validation Subcommittee (EDMVS), which provides guidance to the Agency on

validation of EDSP screening methods and includes experts from government, industry, and stakeholder organizations. This subcommittee also advises the Agency on reducing animal use, modifying procedures to make them less stressful to test animals, and replacing animals where scientifically appropriate. GED is the Agency lead for development of a proposed Tier 2 assay. Dr. Chuck McKenney is leading a team developing a standard multi-generation test using mysids, which are small, marine invertebrates. In addition, Dr. Michael Hemmer and Larry Goodman of GED are working with colleagues from MED on development of a fish life-cycle test under consideration for Tier 2.

Dr. Gerald Ankley of MED is a member of the ecotoxicology Validation Managment Group of the Organization for Economic Cooperation and Development, which is charged with developing and documenting internationally harmonized EDSP screening and testing methods for invertebrates, fish, amphibians, and birds. MED is also the Agency lead for developing two of the proposed Tier 1 assays: a short-term reproduction test with the fathead minnow (*Pimephales promelas*), and metamorphosis assay with the African clawed frog (*Xenopus laevis*). This work is coordinated by Dr. Ankley, Dr. Sig Degitz, and Joe Tietge. In addition, MED is involved in developing full and partial life-cycle tests for Tier 2 assays using fish and frogs. Led by Dr. Patricia Schmieder, MED has also created models for prioritizing chemicals to be screened and tested.

Dr. Robert Kavlock, Director of RTD, is a member of EDMVS. Acting as consultants on various key EDSP activities, several other RTD scientists have presented their research to the EDMVS on a number of protocols, including the male and female puberty assays by Dr. Ralph Cooper; the Hershberger assay and the *in utero* and lactational assay by Dr. Earl Gray; estrogen receptor binding assays and aromatase activity by Dr. Susan Laws; restricted feeding and puberty studies by Dr. Tammy Stoker; steroid formation by Dr. Jerome Goldman; and androgen receptor binding by Dr. Vicky Wilson.

Currently, EPA anticipates that issuance of final guidelines for priority setting and validation of the Tier 1 screen will be completed by 2003. Placement of orders to begin screening of chemicals having high production volume is expected by 2003, and completion of Tier II validation by 2005.

Ozone Sensitivity of Bean Plants Varies Throughout the Development Cycle

Five Western Ecology Division scientists (Drs. David Tingey, E.H. Lee, Wiliam Hogsett, Jilian Gregg, and intern Justin Rodecap) recently published an article showing how ozone sensitivity of the bush bean plant changes as the plant develops. Growing a popular variety of bush beans (*Phaseolus vulgaris*) in open-topped field chambers, the researchers used four different treatments on the plants. They found that ozone exposures during the leaf production stage caused little injury to foliage and had only a small impact on growth and bean yield. Ozone exposures during pod development, however, significantly increased foliar injury and decreased both growth and yield. These results suggest that the greater sensitivity of the bean plants to ozone during their pod-filling and maturation stages is the consequence of reduced energy levels available for the normal processes of repair. Therefore ozone standards set to protect vegetation must consider that ozone sensitivity changes with the stages of plant development. [*Water, Air, and Soil Pollution*, 2002, 39:325-341].

Laboratory Sediment Toxicity Tests Validated in the Field

Amphipods are small, shrimp-like, sediment-dwelling, aquatic animals that are commonly used to test sediment toxicity because of their high sensitivity to many chemicals. Laboratory sediment-amphipod toxicity tests are used extensively to determine the toxicity and bioavailability of chemical contaminants, the extent and severity of pollution impacts, and for setting sediment-quality guidelines. Some critics, however, question their ecological relevance, claiming that responses in a simple, controlled laboratory test cannot be extrapolated to responses in the field. Dr. Steven

Ferraro and Faith Cole of the Western Ecology Division conducted a field validation study of two sediment-amphipod toxicity tests at a Superfund site in Seattle's Elliott Bay, which is contaminated with polycyclic aromatic hydrocarbons (PAHs). They showed that the predictive ability of their toxicity tests was linked to ecologically important field effects, and that, under the proper conditions, the tests can be used for laboratory-to-field extrapolation.

The field validations were conducted by testing relationships between the survival of amphipods in each of two toxicity tests; each of seven observed field effects, including changes in the number of species, abundance, and diversity of sediment-dwelling animals; and total PAHs in the sediments at 30 field stations. Potential confounding environmental variables were identified and accounted for. The findings suggest that when their predictive ability is sufficient, sediment toxicity tests are likely to be more cost-efficient than observational field studies for determining the magnitude and spatial extent of chemical contaminant effects in the field. [*Environmental Toxicology and Chemistry,* 21(7):1423-1437 (2002)].

TECHNICAL ASSISTANCE

Japanese Scientists Visit Three NHEERL Ecology Divisions

Japan is considering extending its chemical management program for the protection of human health to include environmental protection as well. Three Japanese scientists representing the Ministry of the Environment visited several Ecology Divisions between July 29 and August 9, 2002, to meet with NHEERL scientists experienced in the development of toxicity bioassays and applications to ecological risk assessment. These meetings were an important opportunity for international technology transfer of methods and models developed at NHEERL to protect terrestrial, freshwater, and marine ecosystems.

At three Ecology Divisions–Atlantic (AED), Mid-Continent (MED), and Western (WED)–the visitors and NHEERL scientists covered a wide range of topics, including development and application of bioassays, species currently used in bioassays, institutions that conduct bioassays, test organism suppliers, results of chemicals tested, bioassays in ecological risk assessment, sediment toxicity identification and evaluation (TIE) techniques, future challenges for toxicity testing, and applications to ecological risk assessment.

At AED, a series of informal presentations were given on the development of water quality criteria, equilibrium sediment guidelines, and population models to extrapolate from effects on individuals to effects on populations. The Japanese scientists toured the facility, heard a presentation on AED's dissolved oxygen testing system, and viewed the completion of a sediment TIE test.

At MED, additional topics included the development of quantitative structure-activity relationship (QSAR) models to predict the toxic potential of chemicals and to use in ecological risk assessments. The Japanese scientists also participated in a

colloquium on the use of medaka data in risk assessments. Medaka, a fish native to Japan, is one of three fish species used in Japan and internationally to assess the endocrine-disrupting potential of chemical contaminants in the environment. Avian test methods and the use of avian toxicity information in risk assessments were also discussed.

At WED, the interchange focused on standard plant bioassays, various methods for soil invertebrates, and approaches to soil microbial assays used in hazard and risk assessments. Additionally, there were discussions on marine sediment bioassays and tours of the greenhouse, plant growth chambers, and outdoor plots used in plant testing.